REMARKS

Claims 105, 106, 109-131, 133-142, 144-164, 167-171, 184, and 185 are pending in this application. Applicants have amended claims 141, 142, 167, and 168. No new matter has been added.

35 U.S.C. § 112, 2nd Paragraph

The Office Action alleges that claims 141, 142, and 167-169 are indefinite. Applicants have amended claims 141 and 142 to indicate that the mannosidase inhibitor prevents the removal of one or more α 1,2 mannose residue(s) and further prevents the removal of one α 1,3 mannose residue (claim 141) or one α 1,6 mannose residue (claim 142).

Applicants have amended claims 167 and 168 to depend from claim 139 rather than canceled claim 166.

Applicants respectfully request that this rejection of claims 141, 142, and 167-169 be withdrawn.

35 U.S.C. § 103

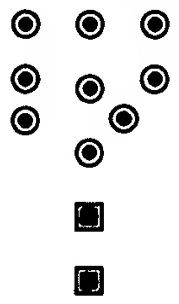
Claims 105, 106, 109-128, 130, 131, 133-138, 184, and 185

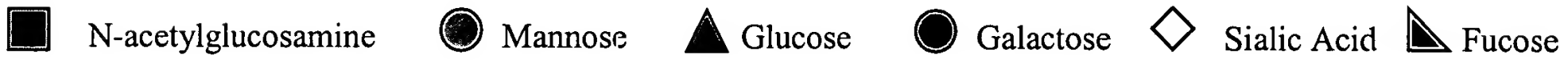
The Office Action alleges that claims 105, 106, 109-128, 130, 131, 133-138, 184, and 185 are obvious in light of Friedman et al. (US Patent No. 5,549,892) and Smith et al. (US Patent No. 5,939,279). Applicants respectfully disagree and submit that a *prima facie* case of obviousness has not been established against these claims.

The claimed invention is directed to methods of producing a preparation of high mannose glucocerebrosidase (hmGCB) that includes a carbohydrate chain having at least four high mannose residues by contacting a cell that expresses human GCB with kifunensine. Contrary to the Examiner's assertions, nothing in Friedman et al. would motivate a skilled artisan to treat a GCB expressing cell with any mannosidase inhibitor to obtain hmGCB. In fact, Friedman et al. teach away from the preparation recited in the claims.

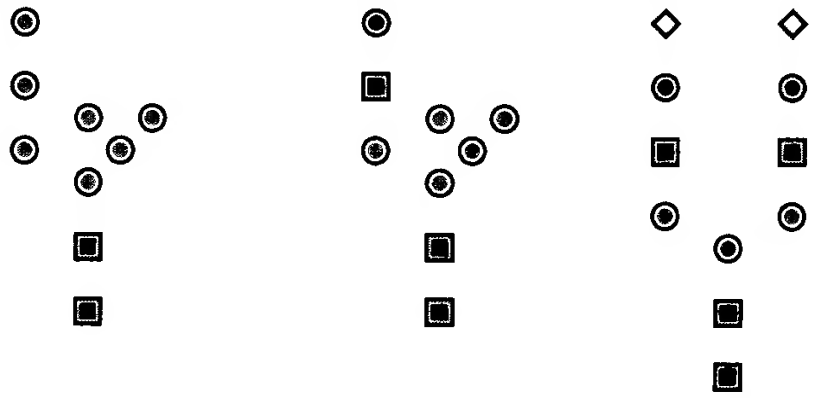
I. Background on N-linked glycan processing in a cell

Generally, N-linked glycans are processed as follows. A precursor oligosaccharide is attached to GCB that includes 9 mannose residues and two N-acetylglucosamine residues.



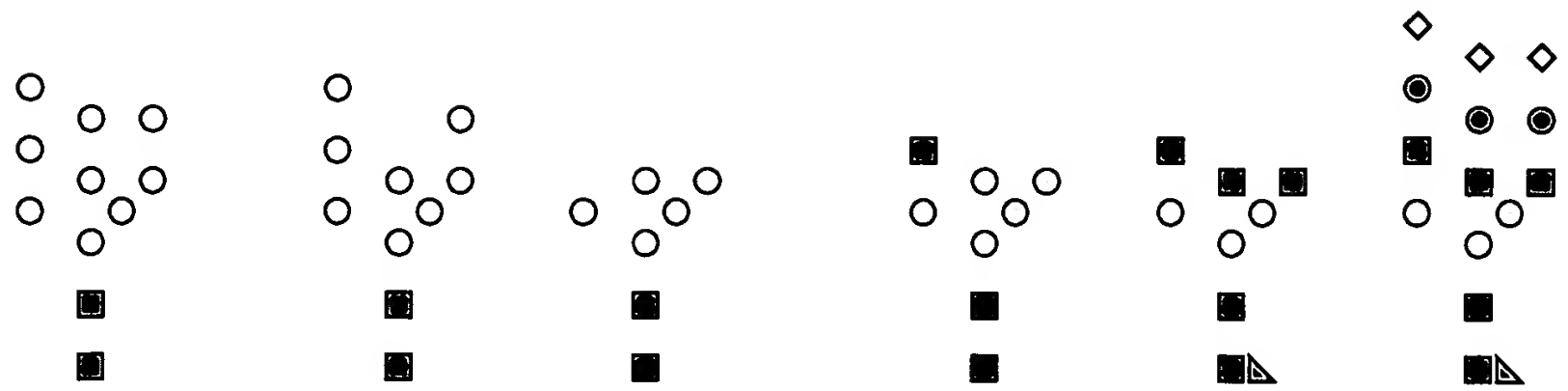


The precursor oligosaccharide is processed in the cell to provide one of three general N-linked glycan structures: a high mannose oligosaccharide, a hybrid oligosaccharide or a complex oligosaccharide. An example of each of these structures is provided below:



High mannose Hybrid Complex

There are three stages of processing of a precursor oligosaccharide that can occur to arrive at these three glycan structures: removal of glucose residues, removal of a variable number of mannose residues, and addition of various sugar residues to the trimmed core (the trimmed core refers to the Man3GlcNAc2 structure). Outlined below is a general scheme of complex oligosaccharide formation.



Briefly, the removal of the glucose residues in the first stage of processing involves removal of all three glucose residues to generate N-linked $Man_9GlcNAc_2$. (This portion of the processing pathway is not indicated above). Processing normally continues to the second stage with removal of mannose residues. Four of the mannose residues of the $Man_9GlcNAc_2$ moiety are bound by α 1,2 linkages. Up to four of these α 1,2-linked mannose residues can be removed by mannosidase IA, IB and IC to generate N-linked $Man_{5\text{-}8}GlcNAc_2$. Protein-linked $Man_5GlcNAc_2$ can then serve as a substrate for GlcNAc transferase I, which transfers a β 1,2-linked GlcNAc residue from UDP-GlcNAc to the core α 1,3-linked mannose residue to form $GlcNAcMan_5GlcNAc_2$. Mannosidase II can then complete the trimming phase of the processing pathway by removing two mannose residues to generate a protein-linked oligosaccharide which contains within it a $Man_3GlcNAc_2$, the "pentasaccharide core". The structure $GlcNAcMan_3GlcNAc_2$ is then a substrate for GlcNAc transferase II, which can transfer a β 1,2-linked GlcNAc residue to the α 1,6-linked mannose residue.

After the trimming phase, monosaccharides are sequentially added to the growing oligosaccharide chain by a series of membrane-bound Golgi glycosyltransferases, each of which is highly specific with respect to the acceptor oligosaccharide, the donor sugar, and the type of linkage formed between the sugars. These can include GlcNAc transferases, galactosyltransferases, sialyltransferases and fucosyltransferases.

Kifunensine acts on glycan processing by preventing the removal of mannose residues on the precursor oligosaccharide. In other words, kifenensine prevents further processing of the

first structure or second structure depicted in the processing pathway provided above. This prevents (or reduces the likelihood of) hybrid and complex oligosaccharides formation.

II. The teachings of Friedman et al. (US Patent No. 5,549,892) would not motivate a skilled artisan to utilize a mannosidase inhibitor to prepare a preparation of GCB

The Office Action alleges at pages 3-4:

> Friedman et al. ... teach the importance of a glycoprotein, human GCB, needed for treatment of Gaucher's disease. They teach the importance of GCB remodeling for the production of a pharmaceutically effective preparation and the encoding human GCB comprises exogenous regulatory and coding sequences (columns 3, 4). Friedman et al. teach that the remodeling of the carbohydrate chains may be accomplished by several different alternative pathways such as utilizing mutant cell lines deficient in certain carbohydrate synthetic pathways (column 6, lines 1-15).

Even if the Friedman reference discloses all of these points, upon reading Friedman et al., a skilled artisan would not be motivated to inhibit the removal of mannose residues from precursor oligosaccharides to obtain a preparation of hmGCB that includes a carbohydrate chain with four or more mannose residues. Friedman et al. explicitly teach away from preparations of GCB that have the requisite carbohydrate structure.

Friedman et al. discuss the remodeling of GCB obtained from placenta (pGCB) and recombinantly produced GCB (rGCB)[1]. (It should be noted that treatment to remove other sugars to expose mannose residues as disclosed by Friedman is not the same as a treatment with a class I mannosidase inhibitor to prevent removal of mannose residues from a precursor oligosaccharide). Throughout the Friedman reference, the "surprisingly" improved pharmacokinetics of remodeled rGCB as compared to remodeled pGCB are reported. See, e.g., column 4, lines 27-45 of Friedman et al. which states

> it was unexpectedly discovered (FIG. 2) that remodelled p-GCR and remodelled r-GCR have different cell type distributions in vivo although the blood clearance of both r-GCR and p-GCR is comparable. Approximately twice as much r-GCR

[1] GCB derived from the placenta is referred to by Friedman as "p-GCR" and recombinantly produced GCB is referred to by Friedman as "r-GCR".

> targets Kupffer cells than does p-GCR. This difference was observed at every time point analyzed (FIG. 2). Table 1 shows that this effect is batch independent. Two batches of r-GCR (batch 1199 and batch 1167) were administered to mice according to the protocol in Example 2 and the animals sacrificed 0.33 hours after administration. The percentage of r-GCR retained in Kupffer cells was found to be twice that of p-GCR. (emphasis added)

Friedman et al. attribute the differences in pharmacokinetics of p-GCB and r-GCB to differences in carbohydrate structure between pGCB and rGCB. Specifically, Friedman et al. state that

> Prior to remodeling, the p-GCB has one high mannose chain plus three complex oligosaccharide chains. In contrast, all four of the carbohydrate chains on the r-GCB are complex. After carbohydrate remodeling, the majority of the branches on the oligosaccharide chains of r-GCB and p-GCB terminate in mannose residues. However, p-GCB retains one high mannose chain, which is absent in the r-GCB.

Thus, Friedman et al. specifically attribute the reduced pharmacokinetics of pGCB to the presence of a high mannose chain that is not present in rGCB. Since Friedman explicitly teach that the presence of high mannose oligosaccharides contribute to the reduced pharmacokinetics of p-GCB, a skilled artisan would clearly not be motivated by this reference to utilize the claimed method that results in an increase in high mannose structures on GCB.

In addition, Friedman et al. attribute the enhanced properties of rGCB to additional carbohydrate residues found on rGCB that are associated with complex or hybrid oligosaccharides and are not present in high mannose oligosaccharides. Specifically, Friedman et al. state at column 4, lines 61-67

> In addition to sequence differences, p-GCR and r-GCR have differences in carbohydrate structures that may contribute to differences in uptake by Kupffer cells. Following the remodelling, the carbohydrate moiety for r-GCR comprises 15-20% oligosaccharide having increased fucose and N-acetyl glucosamine residues compared to remodelled naturally occurring forms such as p-GCR. (emphasis added)

Thus, Friedman et al. teach that the fucose and N-acetylglucosamine content of rGCB are key to the effective targeting and cellular uptake of GCB. As discussed above in section I, addition of fucose and additional N-acetylglucosamine residues to form hybrid and complex oligosaccharides occurs after mannose residues have been removed by mannosidase. Therefore, for argument's sake, even if a skilled artisan would be motivated by Friedman to use a mutant cell line deficient in certain carbohydrate synthetic pathways, the artisan would not be motivated to use a cell line deficient in mannosidase I. These enzymes are essential for processing N-linked glycans to obtain oligosaccharides with fucose and additional N-acetylglucosamine, i.e., hybrid and complex oligosaccharides. See the discussion in section I. Since Friedman et al. contribute the enhanced properties of their GCB preparation to the presence of fucose and N-acetylglucosamine, a skilled artisan reading this reference would not be motivated to inhibit mannosidase I enzymes that are needed to process a precursor oligosaccharide before additional sugars such as fucose and additional N-acetylglucosamine can be added.

III. Smith et al. (US Patent No. 5,939,279) do not make up for the deficiencies of Friedman et al.

The Office Action states that Smith et al.:

> teach that growing eukaryotic cells in the presence of inhibitors of glycoprotein processing can alter N-linked oligosaccharides. They teach that two such inhibitors, deoxymannojirimycin and kifunensine, inhibit α-mannosidases that trim mannoses from $Man_9(GlcAc)_2$ (column 8, lines 4-15). They teach the method of preparing high mannose $Man_9(GlcAc)_2$ glycoproteins by treating HT-29 cells with mannosidase I inhibitors, deoxymannojirimycin or kifunensine ... Therefore, *Smith et al. teach a general method of altering oligosaccharides attached to protein moiety in glycoproteins by growing human cells in the presence of inhibitors of glycoprotein processing*. They teach that the treatment of human HT-29 cells with kifunensine results in glycoproteins comprising $Man_9(GlcAc)_2$. One of such glycoproteins present in HT-29 cells is GCB. (Office Action, page 4) (emphasis in original).

Smith et al. also fail to provide a suggestion or motivation to pursue the subject matter recited in the claims. This reference pertains to bacterial infections. It has absolutely nothing to

do with modifications of proteins to increase their uptake by cells expressing mannose receptors. Smith et al. teach that gram-negative bacteria bind to high-mannose oligosaccharides on host cell surfaces. This is completely unrelated and irrelevant to the subject matter of the claims. Since Friedman et al. clearly teach away from inhibiting mannosidase and from producing GCB with high mannose oligosaccharide structure, a skilled artisan would not be motivated to apply the general methods of Smith et al. to inhibit mannosidase and produce GCB having a carbohydrate chain with four or more mannose residues.

For the reasons discussed above, Applicants respectfully submit that a *prima facie* case of obviousness has not been established against claims 105, 106, 109-128, 130, 131, 133-138, 184, and 185, and request that the obviousness rejection to these claims be withdrawn. In addition, Applicants request that the rejection to claim 129, which depends from claim 105, also be withdrawn.

Claims 129, 139-142, 144-164, and 167-171

The Office Action alleges that claims 129, 139-142, 144-164, and 167-171 are obvious in light of Treco et al. (US Patent No. 6,270,989) in combination with Friedman et al. and Smith et al.

Applicants respectfully traverse this rejection. As discussed above, neither Friedman et al. or Smith et al. would motivate a skilled artisan to use the claimed methods to make a preparation of high mannose GCB. Treco et al. do not mention carbohydrate modifications, mannosidases, or mannosidase inhibitors, such as class I mannosidases at all. Thus, Treco et al. do not make up for the deficiencies of Friedman et al. and Smith et al.

Applicants respectfully request that this rejection be withdrawn because a *prima facie* case of obviousness has not been established against these claims.

Applicants request that this rejection to claim 129 be withdrawn because claim 129 depends from claim 105 and, as discussed above, claim 105 is not obvious because a *prima facie* case of obviousness has not been established against it.

CONCLUSION

Applicants have amended claims 141, 142, 167, and 168. Applicants respectfully submit that the amendments and arguments presented herein have overcome the rejections of the Office Action and that all the pending claims are in condition for acceptance.

Enclosed is a check for the Petition for Extension of Time fee. Please apply any other charges or credits to deposit account 06-1050, referencing Attorney Docket No. 10278-017001.

Respectfully submitted,

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